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Does mating occur among populations of two types in the butterfly *Eurema hecabe* (L.) (Lepidoptera, Pieridae)?

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Abstract In Japan, the butterfly *Eurema hecabe* (L.) consists of two separate groups (subtropical and temperate), and these groups differ in the fringe color (brown or yellow type) of the forewing upperside and are linked with expression of seasonal wing morph and with host-plant utilization. It was recently found that these two types show sympatric distribution on subtropical Okinawa-jima Island, and sexual isolation exists between them. In the present paper, the possibility of mating was investigated among populations of two types in Okinawa-jima I. and its adjacent islands (Ishigaki-jima I. and Honshu) in the laboratory. 1) Mating did not occur between the brown and yellow types of Okinawa-jima I. 2) The brown-type females of Okinawa-jima I. did not mate with the males of Ishigaki-jima I. or Honshu, while 3) the yellow-type females of Okinawa-jima I. mated with the allopatric males of Ishigaki-jima I. or Honshu. However, 4) females of both Ishigaki-jima I. (brown type) and Honshu (yellow type) mated with the males of allopatric populations (Honshu and Ishigaki-jima I.) except those of the brown type of Okinawa-jima I. These results are discussed from the viepoint of evolution of sexual isolation.

Key words *Eurema hecabe*, butterfly, interpopulational mating, sexual isolation, population differetiation.

Reproductive isolation exists among closely related species inhabiting the same geographical area, and prevents or greatly reduces the exchange of genetic material among these species (Mayer, 1963; Futuyma, 1998).

The pierid butterfly *Eurema hecabe* (L.) is widely distributed from the tropical through subtropical to the temperate region. In Japan, this species consists of at least two geographically separate groups (subtropical and temperate) differing in the fringe color of forewing upperside (brown and yellow types), and these two are linked with seasonal expression of wing pattern and the host-plant utilization (Kato and Sano, 1987; Kato and Handa, 1992; Kato *et al.*, 1992). It was recently found that butterflies of these two types show sympatric distribution on Okinawa-jima Island and that they are distinct populations but not different forms within the same species (Kato, 1999, 2000*a*, 2000*b*). Furthermore, sexual isolation seems to exist between these sympatric populations of *E. hecabe* on this Island (unpublished data).

Therefore, it is an important problem to investigate the possibility of mating and hybrid formation between other neighboring populations for understanding of the population differentiation of this butterfly. I here tested the possibility of mating between several other populations of *E. hecabe*.

Materials and methods

Adult females were caught on Ishigaki-jima Island (24.0°N at Ishigaki), Okinawa-jima Island (26.1°N at Naha) and Honshu (34.0°N at Tokyo) and their offspring were used in the experiments.

Individuals of Okinawa-jima I. were classified into the two types according to fringe color (brown or yellow type) of the forewing upperside (Kato, 1999, 2000*a*). Individuals of Ishigaki-jima I. were of the brown type while those of Honshu (Tokyo) are of the yellow one. Larvae were reared under a photoperiod of 16L-8D at 25°C in plastic cups (11 cm in diameter × 6 cm), and fresh cuttings of *Albizzia julibrissin* were given as larval food. Newly eclosed adults were separated according to their sex, and then kept singly in the cup where a cotton-ball soaked with 10% sucrose solution was placed. They were kept under the same condition as the larvae. Adults within one day after adult eclosion was referred to as day-0 adults.

Behavioral experiments were performed from 10 : 00 a. m. to 3 : 00 p. m. during July to September in the laboratory. One virgin female of day-7 was released into a transparent acrylcage (30 cm × 30 cm × 30 cm), and then one sexually active male (day 3 to day 10) of the same population was released to confirm sexual receptivity of the females. Within 30 seconds of the mating of such female with a male, connection of male and female abdominal tips was artificially separated. Third, after pause of 5 minutes, one male of the different population was released into the cage where a receptive female was present, and their mating behavior was observed for 30 min. The cage was placed in an air-conditioned room (25°C) and illuminated with 30 W fluorescent tubes. During illumination the temperature within the cage rose to 27–28°C.

Results

The results are shown in Table 1.

(a) Between the sympatric populations

Between the brown and yellow types of Okinawa-jima I., mating did not occur between the brown female (male) and the yellow male (female). In all cases, females of each type refused the males of the different type although the males courted them.

(b) Between the allopatric populations

Brown-type females of Okinawa-jima I. responded with refusal posture to males of Ishigaki-

Table 1. Mating occurrence among populations of two types in *Eurema hecabe*.

	Location	Fringe color	Male			
			Okinawa-jima I.		Ishigaki-jima I.	Honshu
			Brown	Yellow	Brown	Yellow
Female	Okinawa-jima I.	Brown	—	0/13	0/7	0/11
		Yellow	0/15	—	7/7	11/11
	Ishigaki-jima I.	Brown	0/10	11/11	—	10/10
	Honshu	Yellow	0/10	11/11	10/10	—

Numerals are number of mating pairs/number of pairs tested.

jima I. or Honshu, and no mating took place. In contrast, yellow-type females of Okinawa-jima I. mated with both the males of Ishigaki-jima I. or Honshu. On the other hand, females of Ishigaki-jima I. (brown type) or Honshu (yellow type) mated with males of other populations except the brown-type males of Okinawa-jima I.

In the cases where mating occurred, males actively courted the females and mating occurred within 5 min after releasing of the males. In all the cases where mating did not occur, females showed a refusal posture such as wing flutter or escape in response to courting males. In contrast to the females, males courted even females of the different populations.

Discussion

In the experiments where two sympatric types (brown and yellow) of Okinawa-jima I. were tested, mating did not occur. The females discriminated between the males of the different types. These results coincided with those of the unpublished study where experimental procedures adopted differed.

Between geographically separate populations of Honshu (yellow type) and Ishigaki-jima I. (brown type), females of both populations mated with the males of the different population. Yellow-type females of Okinawa-jima I. also mated with the males of Ishigaki-jima I. (brown type). This suggests that a prezygotic barrier such as sexual isolation is absent between these populations of separate regions. Unpublished observations show that postzygotic barrier seems to exist between two separate populations of Honshu and Ishigaki-jima I. This supports the idea that sexual isolation evolves more easily between the sympatric populations than between allopatric populations (Ehrman, 1965). Between allopatric populations, sexual isolation does not need to develop because these populations do not meet each other, while between two sympatric populations it is needed against the exchange of genetic material and hybrid formation.

Exceptionally, brown-type females of Okinawa-jima I. did not mate with the males of Ishigaki-jima I. (brown type) or Honshu (yellow type). This means that the mate discrimination system is more strict in the brown-type than yellow-type females of this Island, although the reason remains unknown.

For butterflies, mate discrimination by females, but not by males, is common between sympatric species (Tayler, 1972; Silberglied and Tayler, 1978; Wiernasz and Kingsolver, 1992). This is applicable to the populations of *E. hecabe*, in which mating occurrence was based on female behavior.

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摘　　要

日本産キチョウ (鱗翅目，シロチョウ科) 2型の個体群間において交尾は起こりうるか？ (加藤義臣)

日本産キチョウは2つのグループ (亜熱帯と温帯) からなる．それらは互いに前翅表面の縁毛色 (褐色型と黄色型) を異にし，それらの形質は季節型発現や寄主植物の利用とリンクしている．最近，これらの2型が沖縄島に同所的に生息することが明らかになった．沖縄島の2型は同一種内の変異ではなく，別々の集団であり，しかもそれらの間には性的隔離がある．

本実験では，沖縄島および石垣島・本州の個体群間において交尾が起こるかどうかを実験的に調べた．その結果，1) 沖縄島の褐色型の雌 (雄) と黄色型の雄 (雌) は互いに交尾しなかった．2) 沖縄島の褐色型の雌は石垣島 (褐色型) や本州 (黄色型) の雄を拒否し，交尾は成立しなかった．3) 一方，沖縄島の黄色型の雌は石垣島や本州の雄を受け入れて交尾した．4) 石垣島 (褐色型) や本州 (黄色型) の雌はそれぞれ本州や石垣島の雄を受け入れて交尾したが，沖縄島の褐色型の雄とは交尾しなかった．

これらの結果を性的隔離の進化の観点から議論し，同所的集団の方が異所的集団よりも性的隔離が進化しやすいことを結論した．

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